Form Approved Through 09/30/2007 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Progress Report

Review Group	Type	Activity	Grant Number
	5	P01	5 P01 ES009581-10

Total Project Period
From: 5/7/04 Through: 10/31/08

Requested Budget Period
From: 11/1/07 Through: 10/31/08

1. TITLE OF PROJECT
Children's Environmental Health Center

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Name and address, street, city, state, zip code)
Frank D. Gilliland, MD, PhD
University of Southern California
Keck School of Medicine
1540 Alcazar Street, CHP 236
Los Angeles, CA 90033-9013

3. APPLICANT ORGANIZATION (Name and address, street, city, state, zip code)
University of Southern California
2250 Alcazar Street, CSC 219
Los Angeles, CA 90033

2b. E-MAIL ADDRESS
gillilan@usc.edu

4. ENTITY IDENTIFICATION NUMBER
1951642394A1

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Preventive Medicine

2d. MAJOR SUBDIVISION
Keck School of Medicine

5. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Senior Contracts & Grants Administrator
Univ.of Southern California, Dept. of Contracts & Grants
2250 Alcazar Street, CSC 219
Los Angeles, CA 90033

E-MAIL: nihnga@usc.edu

6. HUMAN SUBJECTS
☐ No ☒ Yes
6a. Research Exempt: ☒ No ☐ Yes
6b. Human Subjects Assurance No.: FWA00005906
If Exempt ("Yes" in 6a): Exemption No.
6c. NIH-Defined Phase III Clinical Trial: ☒ No ☐ Yes
If Not Exempt ("No" in 6a): IRB approval date 8/5/07
☐ Full IRB or ☒ Expedited Review

7. VERTEBRATE ANIMALS
☐ No ☒ Yes
7a. If "Yes," IACUC approval Date: 4/27/05
7b. Animal Welfare Assurance No.: A3518-01

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD
8a. DIRECT $466,690
8b. TOTAL $657,936

9. INVENTIONS AND PATENTS
☒ No ☐ Yes If "Yes," ☐ Previously Reported ☐ Not Previously Reported

10. PERFORMANCE SITE(S) *(Organizations and addresses)*
University of Southern California
Keck School of Medicine
Department of Preventive Medicine
1540 Alcazar Street, CHP 236
Los Angeles, CA 90033-9013

11a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*
TEL 323-442-1096
FAX 323-442-3272

11b. ADMINISTRATIVE OFFICIAL NAME *(Item 5)*
Janice Crane
TEL 323-442-2396
FAX 323-442-2835

11c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 14)*
NAME Janice Crane
TITLE Senior Contracts & Grants Administrator
TEL 323-442-2396 FAX 323-442-2835
E-MAIL jcrane@ooc.usc.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 11c. *(In ink. "Per" signature not a*
(b) (6) [redacted]

DATE 9/20/07

Principal Investigator/Program Director (Last, first, middle): Gilliland, Frank D

Project 2 - UCLA Subcontract

DETAILED BUDGET FOR NEXT BUDGET PERIOD -- DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	11/01/07	10/31/08	5P01ES 009581-09

PERSONNEL (Applicant organization only)		Months Devoted to Project			DOLLAR AMOUNT REQUESTED (omit cents)		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Sum. Mnths	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Diaz-Sanchez, David	Principal Investigator	0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
		0.00			$0	$0	$0
SUBTOTALS →							

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD		**$ $96,369**
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS (LAREI)	
	FACILITIES AND ADMINISTRATION COSTS	$34,852
TOTAL DIRECT COSTS FOR NEXT PROJECT PERIOD *(Item 8a, Face Page)*		**$ $131,221**

Principal Investigator/Program Director (Last, First, Middle): Gilliland, Frank D.

BUDGET JUSTIFICATION	GRANT NUMBER
	5P01ES009581-10

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

Dr. Adrian Casilla's has left UCLA as of 9/1/07 to work for Louisiana State University. He is being replaced by Dr. Erina Lin.

CURRENT BUDGET PERIOD	FROM	THROUGH
	11/1/2006	10/31/2007

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

We do not anticipate an excess of 25% to be carried over into the next year.

Principal Investigator/Program Director (Last, First, Middle): Gilliland, Frank D.

PROGRESS REPORT SUMMARY

GRANT NUMBER
5 P01 ES009581-09

PERIOD COVERED BY THIS REPORT

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR	FROM	THROUGH
Gilliland, Frank D.	11/1/2006	10/31/2007

APPLICANT ORGANIZATION
University of Southern California

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Children's Environmental Health Center

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Project 2: Pollution- Enhanced Allergic Inflammation and Phase II Enzymes

A. Specific Aims

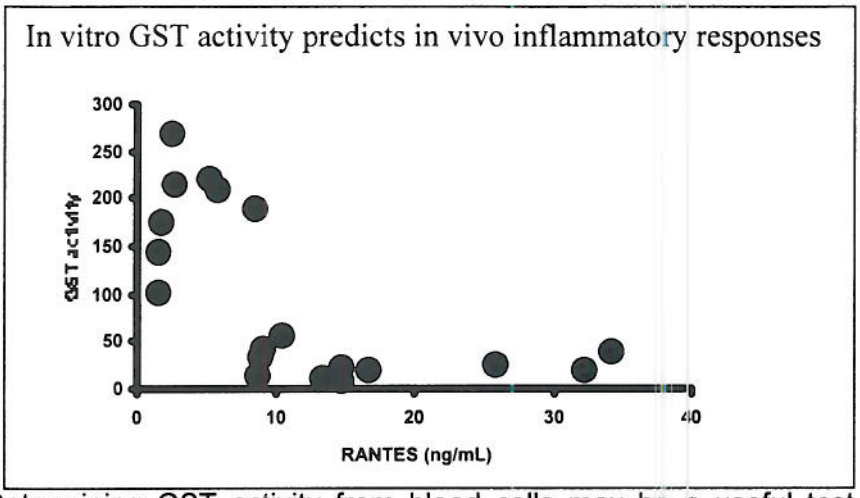
There has been no change in the specific aims of this study, they are to study the role of Phase II enzymes in regulating responses to pollutants in: children's upper airways (Aim #1); the lower airways of healthy and asthmatic individuals (Aim #2) and in mechanistic animal and cellular models of allergic inflammation (Aim #3).

B. Studies and Results

Aim #1: We will test the hypothesis that Phase II enzyme expression in the upper airways are induced by oxidant pollutants and differ between children and adults.

Last year we reported that children appear to be more vulnerable to the adverse effects of oxidant pollutants. Children demonstrated an increased cellular inflammatory response to diesel exhaust particles (DEP). This appeared to be associated with their decreased capacity to produce an adequate Phase II enzyme response to DEP challenge. These results support the concept that the potential of an individual to mount a Phase II antioxidant defense may regulate the development of acute and chronic airway inflammation. This year, we have built on these results to test whether in vitro Phase II enzyme expression can predict in vivo inflammatory responses to DEP. We recruited 20 allergic but otherwise healthy volunteers and obtained blood. We purified peripheral blood mononuclear cells (PBMNCs) from the subjects and stimulated the cells with 10 ug/mL of DEP in 1mL plates. After incubation for 24 hours, glutathione-S-tranferase (GST) activity was measured by following the conjugation of 1mM 1-chloro-2,4,dintrochlorobenzene (CDNB) with 1mM GSH in 200mM sodium phosphate buffer as measured at 340nm using a spectrophotometer over time. Enzyme activity was expressed as millimoles of CDNB conjugated per minute per milligram of cytosolic protein. The subjects were then challenged intranasally with 300ug of DEP with nasal lavages performed prior and 24 hours after challenge. As seen in the figure below, in vitro GST activity predicted in vivo inflammatory responses. The population could be divided into two populations: the first with a GST activity above 100 which had low or minimal inflammatory responses following DEP challenge. In this population (n=8) cell influx into the nose was limited and production of pro-inflammatory cytokines (GM-CSF, IL-1beta) and chemokines (RANTES, IL-8) was not statistically different after DEP challenges vs. baseline levels. The

second population (n=12 had a GST activity under 60 and was characterized by a significant or very robust second inflammatory response following DEP challenge. In this population there was a statistically significant increase in pro-inflammatory cytokines and chemokines after challenge. In addition, GST activity in this population was inversely proportional to cell numbers or RANTES production.



Determining GST activity from blood cells may be a useful test to determine susceptibility to pollutants. Currently, the only test available is by performing in vivo challenge.

Aim #2: **We will test the hypothesis that Phase II enzyme expression in the *lower* airways are induced by oxidant pollutants and differ between asthmatic and non-asthmatic subjects.**
In the past year we have exposed an additional 10 subjects (5 asthmatic and 5 non-asthmatic) to diesel exhaust to study the effect of Phase II expression on lower airway responses. To date we have thus performed exposures on 15 healthy and 20 asthmatic subjects. We have observed that expression of our four sentinel Phase II enzymes (GSTM!, GSTP!, HO-1 and NQO1) is significantly elevated in both healthy and asthmatic subjects following exposure to 2 hours of diesel exhaust (100 ug/m3). No such induction is observed following exposure to either filtered air or nitrogen dioxide. Phase II enzyme expression was measured in cells recovered from induced sputum performed 24 h after exposure. IL-8 levels in this sputum of subjects was significantly inversely correlated with Phase II expression. Thus the higher the levels of GSTP1 the lower the levels of IL-8.

Aim #3: **We will determine the role of Phase II enzymes in regulating the adjuvant effects of particulate pollutants.**
We have previously reported that individuals who lack the ability to make the Phase II enzyme GSTM1 are at increased risk for the pro-inflammatory effects of DEP. Furthermore we have shown that enhancement of Phase II enzymes with sulforaphane can inhibit the production of pro-inflammatory cytokines in respiratory epithelial cells *in vitro*. In order to determine whether GSTM1 itself is important in the regulation of inflammatory response to pollutants, we used siRNA to "knockdown" the GSTM1 gene in bronchial epithelial cells. Expression could be reduced by more than 90% using this methodology. Knockdown of GSTM1 augmented DEP induced cytokine production in these cells. Thus IL-8 levels were almost 3 fold higher in cells where GSTM1 expression was reduced, compared to sham treated cells.

C. Significance

The principal finding of this last year is the close correlation between the capacity of an individual to produce Phase II enzymes and their airway inflammatory response to challenge with DEP. This supports the view that children are more susceptible to high levels of pollutants due to a diminished ability to form this protective antioxidant response. The discovery that in vitro GST expression is associated with in vivo inflammatory responses, provides the potential to develop a diagnostic test for susceptibility to oxidant pollutants.

D. Plans

In the next year we intend to continue recruitment of adults and children for Aims #1 and #2 and further develop an in vitro test to predict airway susceptibility to pollutants.

E. Publications

1. Gilliland, F.D., Li, Y.-F., Gong Jr. H., Diaz-Sanchez, D. Glutathione-S-Transferase *M1* and *P1* Prevent Aggravation of Allergic Responses by Second-hand Smoke. *Am J Resp Crit Care Med* 174:1335-41. 2006
2. Diaz-Sanchez, D., Rumold, R., Gong Jr. H. Challenge with Environmental Tobacco Smoke Exacerbates Allergic Airway Disease in Humans. *J. Allergy Clin. Immunol.* 118:441-446. 2006
3. Wan, J., Diaz-Sanchez D. Association of enhanced IgE production in B cells by diesel exhaust particles and induction of Phase II enzymes. *J.Imunol* 177: 3477-3483. 2006
4. Ritz, S.A., Wan, J., Diaz-Sanchez D. Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. *Am J Physiol Lung Cell Mol Physiol.* 292:L33-9. 2007
5. Wan, J., Diaz-Sanchez D. Antioxidant enzyme induction: a new protective approach against the adverse effects of diesel exhaust particles. *Inhalation Toxicol*
6. Cozen W., Avol E. Diaz-Sanchez D., McConnell R., Gauderman W.J., Cockburn M, Zadnick J., Jyrala M., Mack T.M. Use of an Electrostatic Dust Cloth for Self-administered Home Allergen Collection. *Twin Research and Human Genetics. (in press)*
7. Lin E., Zhang, L., Diaz-Sanchez D. Increased susceptibility of children to the pro-inflammatory effects of diesel exhaust particles due to decreased antioxidant capacity. (*Submitted)*

Principal Investigator/Program Director (Last, First, Middle): Gilliland, Frank D.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Children's Environmental Health Center – Project 2: Pollution-Enhanced Allergic Inflammation and Phase II Enzymes

Total Enrollment: 35 **Protocol Number:**

Grant Number: 5 P01 ES009581-09

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Hispanic or Latino	6	5		11 **
Not Hispanic or Latino	13	11		24
Unknown (individuals not reporting ethnicity)				
Ethnic Category: Total of All Subjects*	19	16		35 *
Racial Categories				
American Indian/Alaska Native		1		1
Asian	7	6		13
Native Hawaiian or Other Pacific Islander				
Black or African American	1	1		2
White	5	4		9
More Than One Race	6	4		10
Unknown or Not Reported				
Racial Categories: Total of All Subjects*	19	16		35 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Unknown or Not Reported	Total
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White	3	2		5
More Than One Race	3	3		6
Unknown or Not Reported				
Racial Categories: Total of Hispanics or Latinos**	6	5		11 **

* These totals must agree.
** These totals must agree.